COMMUNICATIONS

Molecular dynamics of glucose in solution: A quasielastic neutron scattering study

Luis J. Smith[a)]
Argonne National Laboratory, Argonne, Illinois 60439

David L. Price
Centre de Recherche sur les Matériaux à Haute Température, 1D Avenue de la recherche scientifique, 45071 Orléans Cedex 2, France

Zema Chowdhuri
NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

John W. Brady
Department of Food Science, Cornell University, Ithaca, New York 14853

Marie-Louise Saboungi[b)]
Centre de Recherche sur la Matière Divisée, 1B rue de la Férollerie, 45071 Orléans Cedex 2, France

(Received 21 August 2003; accepted 22 December 2003)

The molecular dynamics of glucose dissolved in heavy water have been investigated at 280 K by the technique of quasielastic neutron scattering. The scattering was described by a dynamic structure factor that accounts for decoupled diffusive jumps and free rotational motions of the glucose molecules. With increasing glucose concentration, the diffusion constant decreases by a factor five and the time between jumps increases considerably. Our observations validate theoretical predictions concerning the impact of concentration on the environment of a glucose molecule and the formation of cages made by neighboring glucose molecules at higher concentrations. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1648302]

Simple sugar molecules are known to possess important cryoprotective properties, and are produced in response to desiccation or freezing by a number of species of plants and animals specifically adapted to survive extreme cold or drought.[1] The mechanism of this protective activity has been much debated, with many theories invoking some sort of direct or indirect interaction with nanostructural elements of cells such as proteins and membrane bilayers acting as a water replacement.[2,3] The fact that a number of these sugars, such as the nonreducing disaccharides sucrose and trehalose, have unusually high glass transition temperatures suggests that dynamical properties may also play a contributing role in cryoprotection. In this context, information about the dynamics of sugars under the high concentration regimes associated with such desiccation processes is crucial in the development of theories of cryoprotection. To reach this goal, understanding the fundamental aspects of both the dynamics and the structure of concentrated aqueous solutions of simple sugars is a necessary first step. Numerical simulations of the dynamics and structure of carbohydrate–water systems have been conducted to investigate in detail the change in the water hydrogen-bonding network and the dynamics of the water resulting from sugar addition.[4–8] However, the time scale of the simulations has generally been too small to allow an adequate study of the slow motions of the sugar molecules over significant distances.[7,8] It is therefore important to rely for now on an experimental approach to study the molecular dynamics, more specifically, the temporal and spatial characteristics of the carbohydrate molecular motion.

As the temperature is lowered, the molecular motions occur on a time scale that ranges from picoseconds to seconds and even beyond. Two powerful techniques are available to probe the molecular and microscopic relaxation processes: nuclear magnetic resonance (NMR) and quasielastic neutron scattering (QENS), complemented whenever feasible by neutron spin-echo spectrometry, the ultimate approach being to combine these two techniques in a complementary fashion thereby expanding the time scale. In this work, we have chosen QENS since (1) we can utilize isotopically labeled samples (H and D) to probe dynamics associated with specific sites of the sugar or the solvent on a time scale ranging from picoseconds to nanoseconds, (2) further we can connect a length scale to the time scale by measuring the dynamics over an extended range of wave vector Q from about 0.1 to 1.5 $Å^{-1}$, a clear advantage over the present NMR techniques, and (3) we can complement whenever possible existing NMR results such as those of Moran and

[a)]Present address: Carlson School of Chemistry, Clark University, Worcester, Massachusetts 01610.
[b)]Author to whom correspondence should be addressed. Electronic mail: mls@cnrs-orleans.fr

Jeffrey.[9] While several QENS studies have been made on the water dynamics in mono-[10] and disaccharide[11,12] solutions only a few have attempted to study the sugar dynamics. Magazù *et al.*[11,12] reported that at 323 K, well above the freezing point, the disaccharide molecules diffuse via a random jump diffusion mechanism.

We report here results on QENS experiments on solutions of the monosaccharide glucose in deuterated water at three molar ratios of glucose: D_2O of 1:11, 1:20, and 1:55, corresponding to 48, 33 and 15 wt. %, respectively, of glucose in protonated solutions. The measurements were made at 280 K, a temperature close to the freezing points[9] (267, 270, and 272 K, respectively, for the three concentrations). Glucose is an appropriate subject for these studies since it is the canonical sugar prototype as well as a basic monomeric component of sucrose and trehalose. Since QENS is sensitive mainly to hydrogen, solutions of natural glucose in heavy water were studied in order to accentuate the dynamical signal from the solute molecules compared with the solvent, D_2O. D-glucose (Sigma Aldrich) was first treated with D_2O to replace all five exchangeable hydrogen atoms with deuterium. The sugar was dried and then redissolved in D_2O to reach the desired compositions. Samples were loaded into annular aluminum cans with the annular thickness adjusted for the scattering characteristics of each solution (0.1 mm for the 1:11 and 1:20 samples and 0.5 mm for the 1:55 sample). The very high resolution of the spectrometer used, the High Flux Backscattering Spectrometer (HFBS) at the National Institute of Standards and Technology (NIST), made it possible to investigate the dynamics at 280 K. Quasielastic scattering data were collected with an incident neutron wavelength of 6.271 Å, corresponding to a kinetic energy of 2.08 meV. The spectrometer was set for an energy transfer of ± 36 μeV with a resolution of 1.01 μeV, over a Q range of 0.36 to 1.52 $Å^{-1}$. This energy resolution corresponds to a time scale of 1.3 ns. Data sets were reduced and analyzed with an analysis package provided by the facility.[13]

The scattering was described by a dynamic structure factor that accounts only for the motions of the glucose molecules. At the three concentrations studied, the incoherent scattering (dominant at small Q) from the D_2O amounts to 28%, 13%, and 7%, respectively, of the total incoherent scattering. At higher Q the coherent scattering begins to be significant[14] and the fraction of scattering from the D_2O increases. However, neglect of the quasielastic scattering from the D_2O is justified by the different time scales of the water and sugar motions: in a companion experiment at a time-of-flight spectrometer on glucose solutions at the same concentrations but with deuterated glucose and protonated water,[15] we observed rotational relaxation times on the order of 1–2 ps and diffusion constants on the order of $2-10 \times 10^{-6}\,cm^2/s$ for the water molecules at 280 K. In the Q range investigated here, these values correspond to line shapes with full-widths at half maximum of ~200–1500 μeV for the rotational contribution and full-widths of ~10–300 μeV for the translational contribution. The scattering contribution from the water thus results in a broad, low-intensity signal that does not contribute significantly to the line shape. Assuming that the different kinds of glucose molecular motions are decoupled, the corresponding components of the dynamic incoherent structure factor are convoluted with each other and with the resolution function of the instrument.[16,17] Following the classical study of the water by Teixeira *et al.*,[18] the translational scattering function of the model is represented by a Lorentzian function, where $\Gamma(Q)$ is the half width at half maximum:

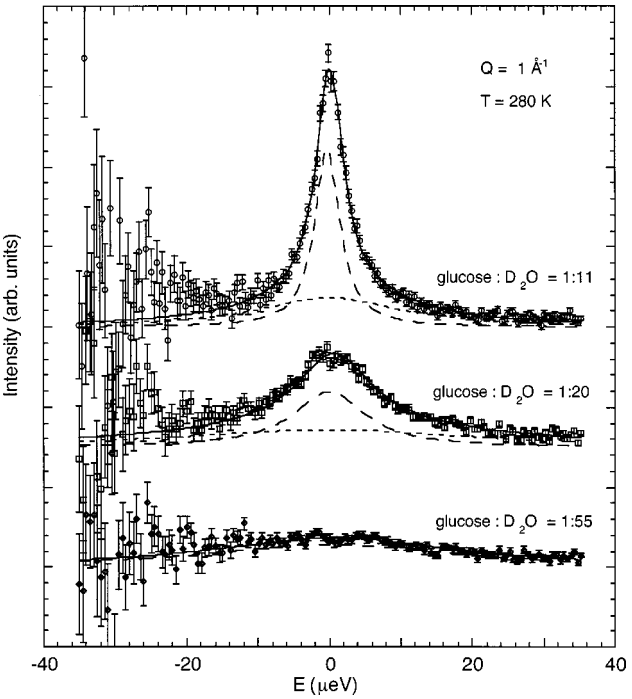

FIG. 1. QENS spectra of glucose/D_2O solutions for the three concentrations at $Q=1\ Å^{-1}$. The solid lines represent the two-Lorentzian fits and the dashed lines the individual Lorentzians.

$$S_{\text{trans}}(Q,E)=\frac{1}{\pi}\frac{\Gamma(Q)}{\Gamma(Q)^2+E^2}. \tag{1}$$

The rotational scattering is modeled on the treatment of Sears[19] for a freely rotating molecule:

$$S_{\text{rot}}(Q,E)=j_0^2(QR)\,\delta(E)+\sum_{k=1}^{\infty}(2k+1)j_k^2(QR) \times\frac{1}{\pi}\frac{k(k+1)\hbar/6\tau_R}{[k(k+1)\hbar/6\tau_R]^2+E^2}, \tag{2}$$

where R is a characteristic molecular radius and τ_R is a relaxation time for rotation. Given the small energy window and low Q range of the experiment, only the first two terms were kept in the expansion. The resulting convolution results in two Lorentzian lines for which the width of the first depends only on the translational motion and that of the second involves both the translational and rotational terms:

$$S_{\text{total}}(Q,E)=j_0^2(QR)\frac{1}{\pi}\frac{\Gamma(Q)}{\Gamma(Q)^2+E^2} +3j_1^2(QR)\frac{1}{\pi}\frac{\hbar/3\tau_R+\Gamma(Q)}{[\hbar/3\tau_R+\Gamma(Q)]^2+E^2}. \tag{3}$$

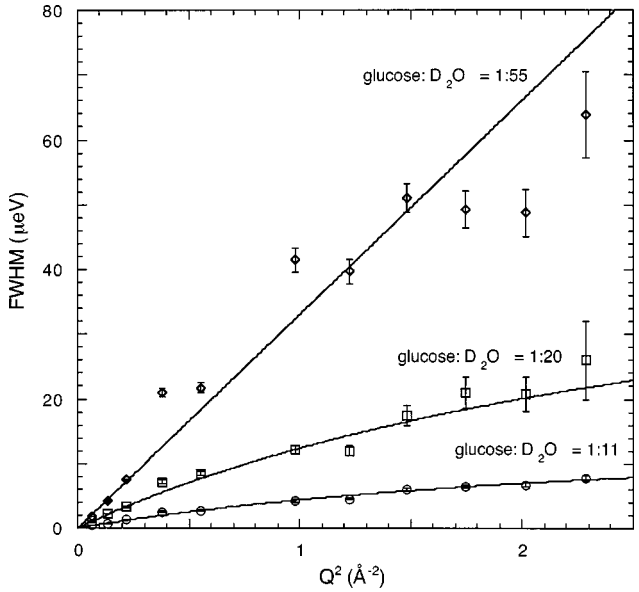

FIG. 2. The full-width at half-maximum of the narrower Lorentzian line vs Q^2 for the three concentrations. The solid lines represent the fits discussed in the text.

Two Lorentzian lines convolved with the instrumental resolution were used to fit the observed spectra for all samples. Since the width of the broader line was larger than the energy window, it was held constant for all Q's. Typical spectra for the three concentrations taken at the same Q, together with the fits of Eq. (3), are shown in Fig. 1. The effect of concentration on the translational diffusion of the glucose is evident: The width of the narrower line decreases with increasing sugar concentration, indicating a slowing down of the diffusive motions. The nature and rate of the diffusive motions of the sugar molecules can be determined from $\Gamma(Q)$. In the case of continuous diffusion (Brownian motion), the FWHM has a quadratic dependence on Q in which the coefficient is related to the diffusion constant, D:[17]

$$2\Gamma(Q)=2\hbar DQ^2. \tag{4}$$

When the molecule can no longer be assumed to be moving via small, fast, elementary jumps, the FWHM variation can be described by the random jump diffusion model.[20] The FWHM is then described by a function that depends on the diffusion constant and a time between jumps, τ_J:

$$2\Gamma(Q)=\frac{2\hbar DQ^2}{1+DQ^2\tau_J}. \tag{5}$$

The Q dependence of the FWHM at the three concentrations shown in Fig. 2 could be fit with both diffusion models. Based on the nonlinear dependence of the FWHM on Q^2 for the 1:11 and 1:20 data, the data for the 1:11, 1:20, and 1:55 solutions were fit with the jump diffusion model. The data for the 1:55 solution was also fit with the continuous diffusion model since the values of the jump times determined from the jump diffusion model were zero within the fitting errors. The fitted values for the diffusion constants, jump times and rotational relaxation times are grouped in Table I.

The FWHMs of the second Lorentzian for the 1:11 and 1:20 solutions were 24 and 50 μeV, yielding 18 and 8.8 ps, respectively for τ_r. The fit for the 1:55 solution did not include a second Lorentzian because the intensity was not significantly greater than zero; its FWHM must be at least 200 μeV if the line is not to have significant intensity in the energy transfer window, corresponding to a rotational correlation time $\geqslant$2 ps which is similar to the values for water as discussed above. Both the glucose rotational relaxation rate and the glucose diffusion constant are thus decreasing strongly with increasing glucose concentration. A substantial increase in the jump time is also evident. Similar diffusion rates for fructose and glucose at high concentrations at similar temperatures have been observed by pulsed field gradient solution NMR. Moran and Jeffrey observed a diffusion rate of 6×10^{-7} cm^2/s for a 40 wt.% (1:15) glucose solution.[9] Rampp *et al.*[21] observed a diffusion rate of 2.8×10^{-7} cm^2/s for a 50 wt.% (1:10) fructose solution at 283 K and a rate of 9.2×10^{-7} cm^2/s for a 30 wt.% (1:23) fructose solution at 277 K. Comparison with the results of Rampp *et al.*[21] suggests that glucose is less restricted in translation at these higher concentrations than fructose, but a direct comparison using similar techniques is still needed.

TABLE I. Diffusion coefficients, jump times, and rotational relaxation times derived from fits with the random jump diffusion (RJD) and continuous diffusion (CD) models.

Concentration	Model	D (10^{-7} cm^2/s)	τ_J (ps)	τ_r (ps)
1:11	RJD	4.5±0.1	78±4	≈18
1:20	RJD	12.3±0.2	25±3	≈9
1:55	RJD	25.2±0.2	0.4±0.6	⩾2
1:55	CD	25.1±0.2	⋯	⩾2

With increasing sugar concentration, the glucose diffusion rate does not simply slow down but its character also changes, as the time between jumps increases from a value close to zero to approximately 80 ps. In numerical simulations of several monosaccharide solutions—particularly at sugar concentrations higher than 29 wt.%—Roberts and Debenedetti[7] observed plateaus in the $\langle r^2\rangle(t)$ plots of the sugar molecules out to the maximum time of the simulations, 2 ns (comparable with the time scale of the present experiment). They associated this behavior with Brownian motions of sugar molecules within a ''cage'' of neighboring sugar molecules. True diffusion would only set in over time scales on which cooperative rearrangements of the cages could occur, beyond the range of both the simulations and the present experiment. This interpretation was supported by their structural analysis of the hydrogen bond network, in which sugar–sugar contributions began to dominate at higher concentrations. Both the trapping times (80 ps) and rms jump lengths, $L=(6D\tau_j)^{1/2}\approx1.4$ Å, that we observe at the highest concentration are consistent with the simulation results and also with values derived for disaccharide molecules by Magazù *et al.*[12] Thus, the large increase in time between jumps and the slowing down of the rotational relaxation at the higher concentrations support the prediction of Brownian motions of the glucose molecules inside cages formed by neighboring glucose molecules at concentrations at and above 1:20.

Discussions with Jean-Marc Zanotti are gratefully acknowledged. D.L.P. thanks the IPNS Division at Argonne for hospitality during the preparation of the manuscript and M.L.S. the MRSEC at the University of Chicago. L.J.S. was supported by the U.S. Department of Energy, Chemical Sciences, under Contract No. W-31-109-Eng-38 and J.W.B. by the National Institutes of Health, Grant No. GM 63018. The neutron facilities at NIST are supported in part by the National Science Foundation under Agreement No. DMR-0086210.

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